## CLAIM AMENDMENTS

1. (Original) A method of generating T-cell lines and clones specific to neisserial proteins, the method comprising isolating peripheral blood mononuclear cells (PBMCs) from the peripheral blood of normal donors and patients recovering from neisserial disease, culturing the PBMCs with neisserial proteins with or without a proliferation stimulant for a prescribed period, stimulating proliferation of T-cell lines and clones which are specific to neisserial proteins, and maintaining same by regular stimulation.

2. (Original) A method as claimed in claim 1, characterised in that the neisserial proteins are prepared from Neisseria meningitidis and/or Neisseria gonorrhoea grown under iron restrictions to induce the expression of iron-regulated proteins.

3. (Previously Presented) A method as claimed in claim 1, characterised in that the peripheral blood is obtained from naturally infected patients at different stages of illness.

4. (Previously Presented) A method as claimed in claim 1, characterised in that the peripheral blood is obtained from naturally infected patients at different stages of illness and the stages include an acute stage (on admission), early convalescence (seven days after admission), late convalescence (six weeks after discharge) and after full recovery (9 months and twelve months after discharge).

5. (Previously Presented) A method as claimed in claim 1, characterised in that the peripheral blood is heparinised or treated with ESTA.

6. (Previously Presented) A method as claimed in claim 1, characterised in that the PBMCs are isolated from the blood by centrifugation.

7. (Previously Presented) A method as claimed in claim 1, characterised in that the PBMCs are initially cultured in medium containing human serum.

8. (Previously Presented) A method as claimed in claim 1, characterised in that the PBMCs are cultured with the neisserial proteins and Interleukin 2 (IL-2) for a predetermined period.

9. (Previously Presented) A method as claimed in claim 1, characterised in that the PBMCs are cultured with the neisserial proteins and Interleukin 2 (IL-2) for a predetermined period and wherein the predetermined period is 3-10 days and may be 5 days.

10. (Previously Presented) A method as claimed in any of claims 1, characterised in that the PBMCs are cultured with the neisserial proteins and Interleukin 2 (IL-2) for a predetermined period and wherein the IL-2 stimulates the proliferation of the activated T-cell lines and clones.

11. (Previously Presented) A method as claimed in claim 1, characterised in that the PBMCs are cultured with the neisserial proteins and Interleukin 2 (IL-2) for a predetermined period and wherein the IL-2 stimulates the proliferation of the activated T-cell lines and clones and said T-cell lines and clones are maintained by weekly stimulation.

12. (Previously Presented) A method as claimed in claim 1, characterised in that the PBMCs are cultured with the neisserial proteins and Interleukin 2 (IL-2) for a predetermined period and wherein the IL-2 stimulates the proliferation of the activated T-cell lines and clones and said stimulation is provided by proteins in the presence of IL-2 and feeder cells.

13. (Previously Presented) A method as claimed in claim 1, characterised in that the PBMCs are cultured with the neisserial proteins and Interleukin 2 (IL-2) for a predetermined period and wherein the IL-2 stimulates the proliferation of the activated T-cell lines and clones and said stimulation is provided by proteins in the presence of IL-2

and feeder cells and said feeder cells are antigen presenting feeder cells and may be autologous Epstein-Barr virus transformed B-lymphocytes (EBVB).

14. (Previously Presented) A method as claimed in claim 1, characterised in that the specificity of the T-cell lines and clones to neisserial proteins is tested prior to storing for example in liquid nitrogen.

15. (Previously Presented) A method as claimed in claim 1, characterised in that the specificity of the T-cell lines and clones to neisserial proteins is tested prior to storing for example in liquid nitrogen and the specificity is tested by measurement of tritiated thymidine incorporation in response to stimulation with neisserial proteins compared to irrelevant antigens.

16. (Previously Presented) A method as claimed in claim 1, characterised in that the specificity of the T-cell lines and clones to neisserial proteins is tested prior to storing for example in liquid nitrogen and the specificity is tested by measurement of tritiated thymidine incorporation in response to stimulation with neisserial proteins compared to irrelevant antigens and wherein said irrelevant antigen is tetanus toxoid.

17. (Previously Presented) A method as claimed in claim 1, characterised in that a phenotype of the T-cell lines and clones are assessed using flow cytometry and specific monoclonal antibodies.

18. (Previously Presented) A method as claimed in claim 1, characterised in that the phenotype of the T-cell lines and clones are assessed using flow cytometry and specific meonoclonal antibodies wherein the antibodies are $CD4^+$, $CD8^-$ and $\alpha/\beta$- and $\gamma/\delta$- T-cell receptor (TCR) specific monoclonal antibodies.

19. (Previously Presented) A method of detecting CD4+ T-cell stimulating proteins, the method comprising fractionating neisserial proteins and testing the ability of said proteins to stimulate proliferation of T-cell lines and clones.

20. (Original) A method as claimed in any of claims 19, characterised in that the proteins are fractionated by SDS-PAGE.

21. (Previously Presented) A method as claimed in any of claims 19, characterised in that the fractions are tested for their ability to stimulate the individual T-cell lines and clones.

22. (Previously Presented) A method as claimed in claim 19, characterised in that fractions containing T-cell stimulants are further characterised by SDS-PAGE.

23. (Previously Presented) A method as claimed in claim 19, characterised in that polyclonal antibodies are raised to the T-cell stimulating fraction proteins.

24. (Previously Presented) A method as claimed in claim 19, characterised in that polycolonal antibodies are raised to the T-cell stimulating fraction proteins and the antibodies are used to screen a genomic menigococcal and/or gonococcal expression library.

25. (Previously Presented) A method as claimed in claim 19, characterised in that polycolonal antibodies are raised to the T-cell stimulating fraction proteins and the antibodies are used to screen a genomic menigococcal and/or gonococcal expression library and wherein the expression library is a λZapII library.

26. (Previously Presented) A method as claimed in claim 19, characterised in that polyclonal antibodies are raised to the T-cell stimulating fraction proteins and isolated neisserial polypeptides which react with the antibodies and their respective DNA fragments are further characterised and sequenced.

27. (Original) A method of detecting $CD4^+$ T-cell stimulating recombinant proteins, the method comprising screening a genomic meningococcal or gonococcal expression library for recombinant proteins which stimulate T-cell lines and clones.

28. (Previously Presented) A method as claimed in claim 27, characterised in that the T-cell lines and clones are meningococcal and/or gonococcal specific T-cell lines and clones.

29. (Previously Presented) A method as claimed in claim 27, characterised in that the genomic meningococcal or gonococcal expression library is a λZapII phage library expressing genomic DNA extracted from a strain of Neisseria meningitidis or a strain of Neisseria gonorrhoea.

30. (Previously Presented) A method as claimed in claim 27, characterised in that the genomic meningococcal or gonococcal expression library is a λZapII phage library expressing genomic DNA extracted from a strain of *Neisseria meningitides* or a strain of *Neisseria gonorrhoea* and *a* representative pool of recombinant pBluescript SKII plasmid are excised from the phage library and transformed into *E. coli* strain XLI-Blue.

31. (Previously Presented) A method as claimed in claim 27, characterised in that the genomic meningococcal or gonococcal expression library is a λZapII phage library expressing genomic DNA extracted from a strain of *Neisseria meningitides* or a strain of *Neisseria gonorrhoea* and a representative pool of recombinant pBluescript SKII plasmid are excised from the phage library and transformed into *E. coli* strain XLI-Blue and the plasmids are excised into XLI-Blue using a helper phage.

32. (Previously Presented) A method as claimed in claim 27, characterised in that the genomic meningococcal or gonococcal expression library is a λZapII phage library expressing genomic DNA extracted from a strain of *Neisseria meningitides* or a strain of *Neisseria gonorrhoea* and a representative pool of recombinant pBluescript SKII plasmid

are excised from th phage library and transformed into *E. coli* strain XLI-Blue and the transformed *E. coli* are cultured in a medium which may contain ampicillin.

33. (Previously Presented) A method as claimed in claim 27, characterised in that meningococcal or gonococcal protein expression is induced by isopropyl-b-Dthio-galactoside.

34. (Previously Presented) A method as claimed claim 27, characterised in that the bacteria are heat-killed and sonicated before adding to antigen presenting cells.

35. (Previously Presented) A method as claimed in claim 27, characterised in that the expressed proteins are tested for their ability to stimulate the individual T-cell lines and clones.

36. (Previously Presented) A method as claimed in claim 27, characterised in that CD4+ T-cell stimulating bacterial cultures are identified and subcultured.

37. (Previously Presented) A method as claimed in claim 27, characterised in that $CD4^+$ T-cell stimulating bacterial cultures are identified and subcultured and the subcultures are rescreened for T-cell stimulation.

38. (Previously Presented) A method as claimed in claim 27, characterised in that $CD4^+$ T-cell stimulating bacterial cultures are identified and subcultured and the $CD4^+$ T-cell stimulants are identified by sequencing and are further characterised.

39. (Previously Presented) A method as claimed in claim 27, characterised in that the genomic meningococcal or gonococcal expression library is a XZapII phage library expressing genomic DNA extracted from a meningococcal or gonococcal genomic lambda phage display library.

40. (Original) A method of detecting CD4+ T-cell stimulating peptides, the method comprising screening meningococcal or gonococcal genomic phage display libraries (PDLs) to identify peptides which stimulate T-cell lines and clones.

41. (Previously Presented) A method as claimed in claim 40, characterised in that the T-cell lines and clones are meningococcal and/or gonococcal specific T-cell lines and clones.

42. (Previously Presented) A method as claimed in claim 40, characterised in that the genomic phage display library (PDL) is generated by fragmenting bacterial DNA, cloning and packaging into bacteriophage vectors.

43. (Previously Presented) A method as claimed in claim 40, characterised in that the genomic phage display library (PDL) is generated by fragmenting bacterial DNA, cloning and packaging into bacteriophage vectors wherein two vectors are used.

44. (Previously Presented) A method as claimed in claim 40, characterised in that the genomic phage display library (PDL) is generated by fragmenting bacterial DNA, cloning and packaging into bacteriophage vectors wherein two vectors are used and the first vector displays peptides up to 1200 amino acids which are expressed at low copy numbers.

45. (Previously Presented) A method as claimed in claim 40, characterised in that the genomic phage display library (PDL) is generated by fragmenting bacterial DNA, cloning and packaging into bacteriophage vectors wherein two vectors are used and wherein one vector displays up to 415 copies of a peptide up to 50 amino acids in size.

46. (Previously Presented) A method as claimed in claim 40, characterised in that the PDLs are amplified in respective Ecoli hosts.

47. (Previously Presented) A method as claimed in claim 40, characterised in that the cells are heat killed before testing for the ability of the peptides to stimulate the T-cell lines and clones.

48. (Previously Presented) A method as claimed in claim 40, characterised in that $CD4^+$ T-cell stimulating PDL cultures are identified and subcultured.

49. (Previously Presented) A method as claimed in claim 40, characterised in that $CD4^+$ T-cell stimulating PDL cultures are identified and subcultured and the subcultures are rescreened for T-cell stimulation.

50. (Previously Presented) A method as claimed in claim 40, characterised in that the $CD4^+$ T-cell stimulants are identified by sequencing and are further characterised.

51. (Previously Presented) A method of detecting CD4+ T-cell stimulating recombinant proteins, using a meningococcal or gonococcal genomic lambda phage display library.

52. (Original) A method as claimed in claim 51, characterised in that the meningococcal or gonococcal genomic lambda phage display library is constructed by cloning randomly amplified PCR products using two random primers, each tagged at 5' end to restriction sites, inserting same into a predigested vector, and plating by infecting *E. coli*.

53. (Previously Presented) A method as claimed in claim 51, characterised in that the meningococcal or gonococcal genomic lambda phage display library is constructed by cloning randomly amplified PCR products using two random primers, each tagged at 5' end to restriction sites, inserting same into a predigested vector, and plating by infecting *E. coli* wherein the vector is a lambda phage.

54. (Previously Presented) A method as claimed in claim 51, characterised in that the meningococcal or gonococcal genomic lambda phage display library is constructed by

cloning randomly amplified PCR products using two random primers, each tagged at 5' end to restriction sites, inserting same into a predigested vector, and plating by infecting *E. coli* wherein the vector is λnRH825 vector.

55. (Previously Presented) A method as claimed in claim 51, characterised in that the meningococcal or gonococcal genomic lambda phage display library is constructed by cloning randomly amplified PCR products using two random primers, each tagged at 5' end to restriction sites, inserting same into a predigested vector, and plating by infecting *E. coli* and the amplified and digested DNA fragments are packaged into the lambda phage using a lambda phage packaging kit.

56. (Previously Presented) A method as claimed in claim 51, characterised in that the meningococcal or gonococcal genomic lambda phage display library is constructed by cloning randomly amplified PCR products using two random primers, each tagged at 5' end to restriction sites, inserting same into a predigested vector, and plating by infecting *E. coli* and the restriction sites are SpeI or NotI.

57. (Previously Presented) A method as claimed in claim 51, characterised in that the meningococcal or gonococcal genomic lambda phage display library is constructed by cloning randomly amplified PCR products using two random primers, each tagged at 5' end to restriction sites, inserting same into a predigested vector, and plating by infecting *E. coli* and the DNA inserts in the plaques formed are sequenced, thereby confirming that the plaques contain DNA fragments of meningococcal or gonococcal origin.

58. (Original) Use of a polypeptide in the manufacture of a vaccine against neisserial disease, the peptide comprising an amino acid sequence as shown in SEQIDNO1 and SEQIDNO2 or an active derivative thereof.

59. (Currently Amended) A The use of the polypeptide as claimed in claim 58, characterised in that the polypeptide is a $CD4^+$ T-cell stimulant.

60. (Original) A DNA construct for use in the manufacture of a medicament for the treatment of neisserial disease the construct comprising a sequence as shown in SEQIDNO3 or an active derivative thereof.

61. (Original) Use of a polypeptide in the manufacture of a vaccine against neisserial disease, the peptide comprising an amino acid sequence as shown in SEQIDNO3 and SEQIDNO4 or an active derivative thereof.

62. (Currently Amended) A use of the polypeptide as claimed in claim 61, characterised in that the polypeptide is a CD4+ T-cell stimulant.

63. (Original) A DNA construct for use in the manufacture of a medicament for the treatment of neisserial disease, the construct comprising a sequence as shown in SEQIDNO1, or an active derivative thereof.

64. (Original) A composition for use as a vaccine against neisserial disease, the composition comprising two peptides with the amino acid sequences as shown in SEQIDNO1 and SEQIDNO2, and SEQIDNO3 and SEQIDNO4 or active derivatives thereof.

65. (Original) A nucleotide sequence comprising a base sequence as shown in SEQIDNO1, or an active derivative thereof, the sequence coding for a polypeptide having an amino acid sequence as shown in SEQIDNO1 and SEQIDNO2, or an active derivative thereof.

66. (Original) A nucleotide sequence comprising a base sequence as shown in SEQIDNO3, or an active derivative thereof, the sequence coding for a polypeptide having an amino acid sequence as shown in SEQIDNO3 and SEQIDNO4, or an active derivative thereof.

67. (Original) A vaccine against neisserial disease, the vaccine comprising polypeptide with some or all of the amino acid sequence as shown in SEQIDNO2 or an active derivative thereof.

68. (Original) A vaccine against neisserial disease, the vaccine comprising polypeptide with some or all of the amino acid sequence as shown in SEQIDNO4 or an active derivative thereof.

69. (Original) A method of treatment of neisserial disease, the method comprising inducing T-cell proliferation with polypeptide comprising one or both of the amino acid sequences shown in SEQIDNO2 and SEQIDNO4, or active derivative(s) thereof.

70. (Original) A purified and isolated DNA composite comprising the sequence shown in SEQIDNO1, or an active derivative thereof.

71. (Original) A purified and isolated DNA composition comprising the sequence shown in SEQIDNO3, or an active derivative thereof.

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